

WHAT IS CLAIMED IS:

1. A method of identifying a compound capable of initiating the signalling of a G-protein coupled receptor (GPCR), which method comprises
 - (a) contacting at least one test compound with cell membrane from at least one GPCR
5 expressing cell or cell line, at least one kinase, and at least one arrestin in a suitable buffer;
 - (b) separating the GPCR bound arrestin from the unbound arrestin; and
 - (c) determining the level of GPCR bound arrestin,whereby a compound which is an agonist of said GPCR is identified when the level of GPCR
10 bound arrestin is raised relative to a control.
2. A method according to claim 1, wherein said kinase is a G-protein coupled receptor kinase (GRK).
3. A method according to claim 1, wherein said arrestin is β -arrestin.
4. A method of identifying a compound capable of initiating the signalling of a G-protein
15 coupled receptor (GPCR), which method comprises
 - (a) contacting at least one test compound with cell membrane from at least one GPCR
expressing cell or cell line and at least one phosphorylation-independent arrestin
mutant;
 - (b) separating the GPCR bound arrestin mutant from the unbound arrestin mutant; and
 - (c) determining the level of GPCR bound arrestin mutant,
20 whereby a compound which is an agonist of said GPCR is identified when the level of GPCR
bound arrestin mutant is raised relative to a control.
5. A method according to claim 4, wherein said arrestin mutant is a β -arrestin mutant.
6. A method according to claim 5, wherein said β -arrestin mutant is R169E- β -arrestin or
25 1-382- β -arrestin.
7. A method according to claim 4, wherein the test compound in step (a) is furthermore brought into contact with a kinase.

8. A method according to claim 7, wherein said kinase is a G-protein coupled receptor kinase (GRK).

9. A method according to claim 1, wherein the arrestin is a labelled arrestin and the determination of the level of GPCR bound arrestin is performed by detecting signals emitted from the formed (arrestin-GPCR) complex; whereby a compound which is an agonist of said GPCR is identified when signals are emitted.

10. A method of identifying a compound capable of initiating the signalling of a G-protein coupled receptor (GPCR), which method comprises

- (a) contacting at least one test compound with cell membrane from at least one GPCR expressing cell or cell line; at least one kinase; and at least one arrestin in a suitable buffer;
 - (b) contacting the resulting mixture with carrier material capable of binding said cell membrane(s); and
 - (c) determining the level of GPCR bound arrestin,
- whereby a compound which is an agonist of said GPCR is identified when the level of GPCR bound arrestin is raised relative to a control.

11. A method according to claim 10, wherein said kinase is a G-protein coupled receptor kinase (GRK).

12. A method according to claim 10, wherein said arrestin is β -arrestin.

13. A method of identifying a compound capable of initiating the signalling of a G-protein coupled receptor (GPCR), which method comprises

- (a) contacting at least one test compound with cell membrane from at least one GPCR expressing cell or cell line and at least one phosphorylation independent arrestin mutant in a suitable buffer;
 - (b) contacting the resulting mixture with carrier material capable of binding said cell membrane(s); and
 - (c) determining the level of GPCR bound arrestin,
- whereby a compound which is an agonist of said GPCR is identified when the level of GPCR bound arrestin mutant is raised relative to a control.

14. A method according to 13, wherein said arrestin mutant is a β -arrestin mutant.
15. A method according to claim 14, wherein said β -arrestin mutant is R169E- β -arrestin or 1-382- β -arrestin.
16. A method according to claim 13 wherein the test compound in step (a) is furthermore
5 brought into contact with a kinase.
17. A method according to claim 16, wherein said kinase is a G-protein coupled receptor kinase (GRK), such as GRK-2.
18. A method according to claim 10, wherein the arrestin is a labelled arrestin and the
10 determination of the level of GPCR bound arrestin is performed by detecting signals emitted from the formed (arrestin-GPCR-carrier) complex; whereby a compound which is an agonist of said GPCR is identified when signals are emitted.
19. A method according to claim 18, wherein the signal emitted by the formed (arrestin-GPCR-carrier) complex is light due to scintillation and the arrestin is radioactively labelled.
20. A method according to claim 19, wherein the carrier material comprises scintillation
15 proximity assay (SPA) beads.
21. A method according to claim 10, wherein the carrier material is provided with wheat germ agglutinate (WGA) to allow binding of cell membrane(s) expressing GPCR(s).
22. A method of identifying a compound capable of deactivating a G-protein coupled receptor (GPCR), which method comprises
20 (a) contacting cell membrane from at least one GPCR expressing cell or cell line with at least one GPCR agonist, at least one kinase and at least one arrestin in a suitable buffer;
- (b) contacting at least one test compound with the resulting mixture to allow said test
25 compound to bind to the GPCR and thereby displace any agonist previously bound thereto;
- (c) separating the GPCR bound arrestin from the unbound arrestin; and
- (d) determining the level of GPCR bound arrestin,

whereby a compound which is an antagonist of said GPCR is identified when the level of GPCR bound arrestin is lowered relative to a control.

23. A method according to claim 22, wherein said kinase is a G-protein coupled receptor kinase (GRK), such as GRK-2.

5 24. A method according to claim 22, wherein said arrestin is β -arrestin.

25. A method of identifying a compound capable of deactivating a G-protein coupled receptor (GPCR), which method comprises

- 10 (a) contacting cell membrane from at least one GPCR expressing cell or cell line with at least one GPCR agonist and at least one phosphorylation independent arrestin mutant in a suitable buffer;
- (b) contacting at least one test compound with the resulting mixture to allow said test compound to bind to the GPCR and thereby displace agonist previously bound thereto;
- (c) separating the GPCR bound arrestin mutant from the unbound arrestin mutant; and
- 15 (d) determining the level of GPCR bound arrestin,
- whereby a compound which is an antagonist of said GPCR is identified when the level of GPCR bound arrestin is lowered relative to a control.

26. A method according to claim 25, wherein said arrestin mutant is a β -arrestin mutant.

20 27. A method according to claim 26, wherein said β -arrestin mutant is R169E- β -arrestin or 1-382- β -arrestin.

28. A method according to claim 25 wherein the test compound in step (a) is furthermore brought into contact with a kinase.

29. A method according to claim 28, wherein said kinase is a G-protein coupled receptor kinase (GRK), such as GRK-2.

25 30. A method according to claim 22, wherein the arrestin is a labelled arrestin and the determination of the level of GPCR bound arrestin is performed by detecting signals emitted

from the formed (arrestin-GPCR) complex; whereby a compound which is an antagonist of the GPCR used is identified when a reduction in signal is detected.

31. A method of identifying a compound capable of deactivating a G-protein coupled receptor (GPCR), which method comprises

- 5 (a) contacting cell membrane from at least one GPCR expressing cell or cell line with at least one GPCR agonist, at least one kinase and at least one arrestin in a suitable buffer;
- (b) contacting at least one test compound with the resulting mixture to allow said test compound to bind to the GPCR and thereby displace any agonist previously bound
- 10 thereto;
- (c) contacting the mixture resulting from (c) with carrier material capable of binding said cell membrane(s); and
- (d) determining the level of GPCR bound arrestin,
- whereby a compound which is an antagonist of said GPCR is identified when the level of
- 15 GPCR bound arrestin is lowered relative to a control.

32. A method according to claim 31, wherein the kinase is a G-protein coupled receptor kinase (GRK), such as GRK-2.

33. A method according to claim 31, wherein the arrestin is β -arrestin.

34. A method of identifying a compound capable of deactivating a G-protein coupled receptor (GPCR), which method comprises

- 20 (a) contacting cell membrane from at least one GPCR expressing cell or cell line with at least one GPCR agonist and at least one phosphorylation independent arrestin mutant in a suitable buffer;
- (b) contacting at least one test compound with the resulting mixture to allow said test
- 25 compound to bind to the GPCR and thereby displace agonist previously bound thereto;
- (c) contacting the mixture resulting from (c) with carrier material capable of binding said cell membrane(s); and
- (d) determining the level of GPCR bound arrestin,
- 30 whereby a compound which is an antagonist of said GPCR is identified when the level of GPCR bound arrestin is lowered relative to a control.

35. A method according to claim 34, wherein said arrestin mutant is a β -arrestin mutant.
36. A method according to claim 35, wherein said β -arrestin mutant is R169E- β -arrestin or 1-382- β -arrestin.
37. A method according to claim 34 wherein the test compound in step (a) is furthermore
5 brought into contact with a kinase.
38. A method according to claim 37, wherein said kinase is a G-protein coupled receptor kinase (GRK), such as GRK-2.
39. A method according to claim 31, wherein the arrestin is a labelled arrestin and the
10 determination of the level of GPCR bound arrestin is performed by detecting signals emitted from the formed (arrestin-GPCR-carrier) complex; whereby a compound which is an antagonist of the GPCR used is identified when a reduction in signal is detected.
40. A method according to claim 39, wherein the signal emitted by the formed (arrestin-GPCR-carrier) complex is light due to scintillation and the arrestin is radioactively labelled.
41. A method according to claim 40, wherein the carrier material comprises scintillation
15 proximity assay (SPA) beads.
42. A method according to claim 31, wherein the carrier material is provided with wheat germ agglutinate (WGA) to allow binding of cell membrane(s) expressing GPCR(s).
43. A method according to claim 1, which is a high throughput screening method.
44. Use of a compound identified according to a method as defined in any of claims 1 to 43
20 as a therapeutically effective substance.
45. Use of a compound identified according to a method as defined in any of claims 1 to 43 as a lead compound in drug design, wherein structure and/or biological properties of said compound are modified in order to provide a therapeutically effective substance.

46. A method for producing a pharmaceutical preparation comprising (i) identifying a compound using a method according to claim 1, and (ii) mixing the identified compound with a pharmaceutically acceptable carrier.

5 47. A method for producing a pharmaceutical preparation comprising (i) modifying the structure and/or biological properties of a compound identified using a method according to claim 1, (ii) mixing the modified compound with a pharmaceutically acceptable carrier.

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